# ANALYZING DIGITAL HOLOGRAPHIC MICROSCOPY DATA FOR HEMATOLOGY APPLICATIONS

## CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** This application is a continuation of U.S. application Ser. No. 15/318,831 filed Dec. 14, 2016, which is a national phase filing under 35 U.S.C. § 371 of international patent application no. PCT/US2015/035945 filed Jun. 16, 2015, which claims the benefit of U.S. provisional application Ser. No. 62/012,636 filed Jun. 16, 2014, which is incorporated herein by reference in its entirety.

## TECHNICAL FIELD

**[0002]** The present disclosure relates generally to analyzing digital holographic microscopy (DHM) for hematology applications. The various systems, methods, and apparatuses described herein may be applied to, for example, red blood cell (RBC) volume measurement and white blood cell (WBC) differential (cell type classification) applications.

## BACKGROUND

**[0003]** Digital holographic microscopy (DHM), also known as interference phase microscopy, is an imaging technology that provides the ability to quantitatively track sub-nanometric optical thickness changes in transparent specimens. Unlike traditional digital microscopy, in which only intensity (amplitude) information about a specimen is captured, DHM captures both phase and intensity. The phase information, captured as a hologram, can be used to reconstruct extended morphological information (such as depth and surface characteristics) about the specimen using a computer algorithm. Modern DHM implementations offer several additional benefits, such as fast scanning/data acquisition speed, low noise, high resolution and the potential for label-free sample acquisition.

**[0004]** Conventional cellular analysis techniques such as volume measurement and classification rely on two-dimensional cellular images that lack topographical information. Thus, while these techniques may analyze a cell based on information such as intensity, their accuracy is limited due to a lack of knowledge of the size and shape of the cell. Accordingly, it is desired to provide cellular analysis techniques which are applicable to imaging modalities such as DHM that provide more detailed information regarding cellular structure.

## SUMMARY

**[0005]** Embodiments of the present invention address and overcome one or more of the above shortcomings and drawbacks, by providing methods, systems, and apparatuses related to analyzing digital holographic microscopy (DHM) for hematology applications. Additionally, as explained in further detail in the disclosure, the technology described herein may be applied to other clinical applications as well.

**[0006]** The ability of DHM to achieve high-resolution, wide field imaging with extended depth and morphological information in a potentially label-free manner positions the technology for use in several clinical applications. For example, in the area of hematology DHM may be used for red blood cell (RBC) volume measurement, white blood cell (WBC) differential (cell type classification). For urine sediment analysis DHM allows for scanning a microfluidic sample in layers to reconstruct the sediment (possibly without waiting for sedimentation); improving the classification accuracy of sediment constituents. DHM may also be used for tissue pathology applications through utilization of extended morphology/contrast of DHM (e.g. to discriminate cancerous from healthy cells, in fresh tissue, without labeling). Similarly, for rare cell detection applications may utilize extended morphology/contrast of DHM (e.g. to differentiate rare cells such as circulating tumor/epithelial cells, stem cells, infected cells, etc.).

**[0007]** According one aspect of the present invention, as described in some embodiments, a method for analyzing digital holographic microscopy (DHM) data for hematology applications includes receiving DHM images acquired using a digital holographic microscopy system. One or more connected components are identified in each of the plurality of DHM images. One or more training white blood cell images are generated from the one or more connected components and a classifier is trained to identify white blood cell types using the one or more training white blood cell images. When a new DHM image is received, a new white blood cell image is extracted from the DHM image. Then classifier may then be applied to the new white blood cell image to determine probability values, with each respective probability value corresponding to one of the white blood cell types. The new white blood cell image and the plurality of probability values may then be presented in a graphical user interface. In some embodiments, a complete blood cell (CBC) test may be performed using the probability values.

**[0008]** Various enhancements, modifications, or additions may be made to the aforementioned in different embodiments of the present invention. For example, in some embodiments, prior to identifying the one or more connected components, a thresholding is applied to the each of the plurality of DHM images to highlight bright spots in each respective DHM image. Components having a size below a predetermined threshold value (i.e., small connected components) may then be removed. In some embodiments, the classifier is a K-Nearest Neighbor (K-NN) classifier. Such a classifier may use texton-based texture features extracted from each of the plurality of DHM images to classify the new DHM image. In other embodiments, the classifier is a visual vocabulary dictionary (e.g., vocabulary histogram) trained using hierarchical k-means and a scale-invariant feature transform (SIFT) descriptor as a local image feature. For example, dense SIFT descriptors may be extracted from each of the plurality of DHM images and used to construct a binary search tree representative of a vocabulary dictionary structure. The visual vocabulary dictionary can then be generated based on the binary search tree. A one against one n-label supporting vector machine (SVM) may be used for identifying one or more of the plurality of white blood cell types in the DHM images. In still other embodiments, the classifier is a deep learning classifier trained using an auto-encoder convolutional neural network (CNN).

**[0009]** Additionally, in some embodiments of the aforementioned method, a digital staining technique is applied to the white blood cell images. For example, in one embodiment, a mapping is determined between optical density and coloring associated with a staining protocol. Optical density information associated with the new white blood cell image is also determined. Prior to presenting the new white blood